Within-clutch patterns of yolk testosterone vary with the onset of incubation in black-headed gulls

Mueller, Wendt; Eising, CM; Dijkstra, C; Groothuis, TGG

Published in:
Behavioral Ecology

DOI:
10.1093/beheco/arh091

Behavioral Ecology Vol. 15 No. 6: 893–897
doi:10.1093/beheco/arh091
Advance Access publication on June 16, 2004

Within-clutch patterns of yolk testosterone vary with the onset of incubation in black-headed gulls

Wendt Müller, Corine M. Eising, Cor Dijkstra, and Ton G. G. Groothuis
Department of Animal Behavior, Kerklaan 30, P.O. Box 14, University of Groningen, 9750 Haren, Groningen, The Netherlands

Hatching asynchrony in birds produces an age and size hierarchy among siblings. Later-hatching chicks have a competitive disadvantage, and brood reduction may occur when food availability is insufficient to raise all chicks. When early-hatched chicks fail to survive or if the circumstances allow raising all chicks, mothers should reverse the disadvantage to later-hatched chicks. Increasing deposition of maternal androgens with the laying sequence has been suggested to compensate for detrimental effects of hatching asynchrony, allowing a more precise adjustment of the survival probabilities of each chick. Here, we show for black-headed gulls that the increase in yolk testosterone with each successive egg is greater when the mother incubates longer before clutch completion, which is the major determinant of the degree of hatching asynchrony. This finding supports the idea that yolk testosterone has a compensatory function in the context of hatching asynchrony. Our data further show that if the time needed to complete a clutch is lengthened, the developmental differences due to incubation between the first- and the last-laid eggs increase. In addition, the onset of incubation before clutch completion occurs sooner as the breeding season progresses. Both long inter-egg intervals and the seasonal shift in incubation behavior enhance the necessity of compensation for later-hatching chicks. Indeed, yolk levels of testosterone increased more steeply over the laying order, if the duration of the egg-laying period was extended and in later-laid clutches. We suggest that prolactin plays a key role in the adjustment of testosterone allocation to the incubation pattern. *Key words:* hatching asynchrony, maternal effects, yolk androgens. *[Behav Ecol 15:893–897 (2004)]*

Hatching asynchrony, as reported for a variety of avian species, results in broods that show a hatching-order-dependent size hierarchy among siblings (Clark and Wilson, 1981; Stockland and Amudsen, 1988). Due to the competitive disadvantage of later-hatched chicks, they are less likely to survive (Mock et al., 1990; O'Connor, 1978). Several hypotheses have been proposed to explain the adaptive value of hatching asynchrony, of which Lack's brood reduction hypothesis was the first and most prominent (Lack, 1947). This hypothesis proposes hatching asynchrony as a means of adjusting brood size when food availability during the nestling stage is insufficient to raise the entire brood. However, experimental tests have provided little support for this, and several other hypotheses have been put forward to explain hatching asynchrony (Stoleson and Beisssinger, 1995). In Herring Gulls *Larus argentatus*, survival of the last-hatched chick has been shown to be extremely rare unless one of the older siblings fails to hatch or survive (Graves et al., 1984). The last-laid egg has therefore been suggested to be an insurance against hatching failure or early loss of the first- or second-laid egg or its chick (Forbes et al., 1997; Graves et al., 1984; Stoleson and Beissinger, 1995). In case of no such failure, hatching asynchrony should facilitate the reduction to the optimal brood size.

The main mechanism for how birds induce hatching asynchrony is the timing of the onset of incubation before clutch completion (e.g., Mead and Morton, 1985). However, birds, especially open field breeders such as gulls, having high predation rates of unattended eggs (e.g., Brouwer and Spaans, 1994; Parsons, 1972) and the risk of sun radiation that might lower the viability of their eggs (Webb, 1987), might face a constraint in the onset of incubation. Thus, they might be forced to an early onset of incubation to avoid egg predation or a decline in egg viability (Bollinger et al., 1990; Dunlop, 1910; Parsons, 1976; Webb, 1987). This may preclude a complete control of the hatching pattern, thereby calling for additional mechanisms that allow the shaping of the optimal brood size.

The transfer of maternal hormones into the eggs of avian species has been suggested to provide such a mechanism. Maternal hormones have been shown to vary systematically with the laying order within a clutch (e.g., French et al., 2001; Gil et al., 1999; Groothuis and Schwabl, 2002; Lipar et al., 1999; Royle et al., 2001; Schwabl et al., 1997) and also affect embryonic development. In particular, increased yolk androgens enhance the development of the hatching muscle, which is needed for breaking the eggshell (Lipar, 2001; Lipar and Ketterson, 2000) and leads to earlier hatching (Eising et al., 2001). Therefore, enhanced allocation of yolk androgens to later-laid eggs might reduce asynchrony in hatching and hierarchies within nests. Furthermore, yolk androgens promote competitiveness of the chick in the nestling stage (Eising and Groothuis, 2003; Schwabl, 1993, 1996). Thus, yolk androgens may serve as a mechanism to mitigate detrimental effects of hatching asynchrony for later-hatched chicks in a brood.

This leads to our following prediction: Mothers should adjust the hormone allocation to her incubation pattern to obtain a certain degree of hatching asynchrony. In other words, the steepness of the increase in androgen content with egg laying sequence should increase with an earlier onset of incubation because the latter leads to a larger degree of hatching asynchrony (e.g., Mead and Morton, 1985).

We tested this prediction for the black-headed gull *Larus ridibundus*, a species with a fixed clutch size, which typically

Address correspondence to W. Müller. E-mail: w.mueller@biol.rug.nl.
Received 26 May 2003; revised 7 January 2004; accepted 12 January 2004.

hatches asynchronously (Cramp and Simmons, 1983). As an open field breeder, black-headed gulls face the typical high egg predation of *Larus* gulls (e.g., Dunlop, 1910). In addition, a clear increase in yolk androgens within the laying order has been described previously (Eising et al., 2001; Groothuis and Schwabl, 2002).

In addition, we looked at two other factors that may influence hatching asynchrony and androgen allocation to the eggs. First, the degree of hatching asynchrony might not only depend on the onset of incubation, but also on the time intervals between laying of the subsequent eggs, which vary considerably between individuals of the same species (e.g., MacRoberts and MacRoberts, 1972). Given the need for early onset of incubation, an increasing time span between the laying of the first and last eggs potentially enhances the degree of hatching asynchrony. This makes a compensatory process such as elevated androgen levels in the last laid-eggs particularly important.

Second, in several avian species the tendency to start incubation earlier after laying of the first egg increases in the course of the breeding season (e.g., Beukeboom et al., 1988; Meijer et al., 1990; Sharp et al., 1979). Both could be responsible for the enhanced hatching asynchrony later in the year in species with a constant clutch size (e.g., Courtney, 1979; Hebert and McNeil, 1999). Therefore, the androgen allocation to the last-laid egg should increase over the time of year to compensate for the increased disadvantage of the last-hatched chick in nests of late breeders.

MATERIALS AND METHODS

Study species and egg collection

Black-headed gulls are monogamous, colonial breeders. The clutch typically consists of three eggs, which are laid over a three- to five-day period (Cramp and Simmons, 1983). In 2000 and 2001, nests of several neighboring black-headed gull colonies (300–1000 breeding pairs) along the northeast coast of the Netherlands were checked once a day for egg laying. In 2001 we also collected late clutches that were laid after the whole colony area was flooded when the first chicks had just hatched. These clutches, therefore, are most likely replacement clutches (laying date >165). Freshly laid eggs were marked with a non-toxic marker referring to the position within the laying order and the date of laying (day of the year). To obtain as accurate laying dates as possible nests were visited between 1000–1100 h each day, because laying normally takes place during the early morning. After clutch completion all eggs of the clutch were collected on the same day and the eggs were weighed to the nearest 0.1 g. Based on the laying date, the laying interval was defined as the difference in laying date between last and first egg (laying interval = 2: all three eggs are laid on consecutive days).

Estimation of hatching asynchrony

To obtain an estimation of both whether and how long the eggs had been incubated prior to clutch completion and the hormone data of the same clutch, we incubated all eggs artificially (37.5°C with 60% humidity) for a standard time to obtain measurable embryo weights. The incubation procedure differed slightly between years. In 2000 all eggs of a clutch were incubated for 72 h and subsequently stored at −20°C. However, we observed that sometimes the developmental stage of the eggs varied quite markedly within a clutch, indicating that incubation had started well before clutch completion. To avoid effects of differences in incubation time on yolk levels of androgens, in 2001 we tried to compensate for these differences by incubating last-laid eggs for one day longer than first-laid eggs. By doing so we tried to equalize the developmental stage of the first-laid and the last-laid eggs within a clutch. This also reduced a potential effect of incubation on hormone levels. In a later study, we found for our study species that hormone levels drop from day 0 to day 1 of incubation and subsequently remain stable until day 8 (Eising CM, unpublished data; see also Elf and Fivizanni, 2002 for similar data on the domestic chicken). Endogenous production of androgens, another potential confounding factor, does not start before day five of development (Woods et al., 1975), and there are no indications that endogenous androgens are transferred into the yolk (Elf and Fivizanni, 2002).

Because the mean incubation time in our study was 4.2 ± 0.09 days and all our eggs were incubated for more than one day, differences in the hormone levels of subsequent eggs in a clutch cannot be explained by incubation time and/or endogenous production.

For the analysis, eggs were defrosted and the yolk and embryo separated. The embryos were weighed to an accuracy of 0.1 μg. Based on a dataset of embryo weights resulting from known incubation times of 0–13 days ($N = 137$; Eising CM, unpublished data), we derived a formula to calculate incubation times from embryo weights using the curve estimation function in SPSS 11.0, 2002 (see Parsons 1972 for a similar approach). The following formula was obtained: incubation time = 3.3652 + (4.8382 × embryoweight) + [−0.6073 × (embryo weight)^2], (df = 134, $r^2 = .91$). This formula was subsequently used to estimate the incubation time of our samples on the basis of embryo weight. We decided not to use published formulas (e.g., Ricklefs, 1987) because these estimations were done for embryos of much older age and there were large differences between species. Because embryos do not have any androgen receptors before they are at least one week old (see, e.g., Godsave et al., 2002), embryonic development at that stage cannot be influenced by maternal yolk androgens. Therefore, embryonic development is very likely to depend mainly on maternal heat transfer.

We were interested in the duration of incubation prior to clutch completion, as this determines the degree of hatching asynchrony. Therefore we subtracted from the estimated incubation duration the time that the eggs had been incubated artificially. The difference in natural incubation time between the last-laid egg and the first-laid egg was taken as an estimate for how long maternal incubation had taken place before the last egg was laid. This estimated onset of incubation before clutch completion (subsequently OIC) was used for the statistical analysis.

Hormone analysis

The yolks were homogenized with 1 ml water per gram of yolk. About 150 mg of the yolk/water emulsion was used for hormone analysis, keeping all eggs of a clutch in the same assay. Each assay contained clutches of the two different laying date categories. We followed a standard procedure according to Schwabl (1993), with a slight modification. Briefly, samples were extracted twice with 4 ml petroleum ether/diethylether (30/70%), followed by precipitation with 90% ethanol to remove neutral lipids. Subsequently, the hormones were separated on diatomaceous earth chromatography columns. Androgen concentrations were measured in double competitive-binding radioimmunoassays (RIA) with tritiated hormone (NEN, the Netherlands) and hormone-specific antibodies (Endocrine Science, USA). The average recovery was 49.2% for testosterone and 58.6% for androstenedione. The inter-assay coefficients of variation were 15.3% for

Table 1

Embryo size (g) and testosterone and androstenedione (A_4) concentrations (pg/mg) for first-laid (A-egg) and last-laid (C-egg) eggs (mean ± SE)

Variable	2000 ($N = 11$)	2001 (early) ($N = 31$)	2001 (late) ($N = 11$)
Testosterone A (pg/mg)	7.11 ± 1.05	15.90 ± 1.38	14.46 ± 3.40
Testosterone C (pg/mg)	10.53 ± 1.23	19.78 ± 1.47	32.78 ± 5.30
A_4 A (pg/mg)	338.59 ± 54.82	557.63 ± 77.25	434.60 ± 93.5
A_4 C (pg/mg)	896.90 ± 187.15	968.78 ± 149.25	654.65 ± 133.77
Embryo size A (g)	0.39 ± 0.10	0.16 ± 0.02	0.36 ± 0.07
Embryo size C (g)	0.08 ± 0.04	0.05 ± 0.01	0.13 ± 0.04

Subdivided according to year and laying date category: early < 140 (day of the year), 2000: $N = 22$, 2001: $N = 31$, except for A_4: $N = 23$; late > 163 (day of the year), 2001: $N = 11$.

testosterone and 19.2% for androstenedione; for testosterone, intra-assay variation was 12.6% and for androstenedione it was 18.6%.

As a measure of the steepness of hormone change over the laying order of a clutch we used $(H_{\text{last-laid egg}} - H_{\text{first-laid egg}})/H_{\text{first-laid egg}}$, where H_x = yolk hormone titer of egg x. This corrected for between-clutch variation in hormone levels, as we were interested in within-clutch variation. For all statistical analyses we used this obtained relative value for the increase of testosterone with laying order.

Statistical analyses

All variables were checked for normality using the Kolmogorov-Smirnov test. Subsequently, variables were tested using parametric statistics (Pearson correlation) or univariate linear regression. All tests were carried out using SPSS 11.0, 2002.

RESULTS

Sixty-four clutches (22 in 2000, 42 in 2001) were included in the analysis. The onset of incubation (OIC) varied from 0.24 to 5.05 days before laying of the last egg of the clutch. Hormone concentrations of testosterone and androstenedione were within the range of a previous study (Eising et al., 2001; for details see Table 1), except for last-laid eggs of late clutches, but clutches of this laying date category were not included in the previous study. Due to extraction failure of either the first- or the last-laid egg for androstenedione, only 56 of the 64 clutches could be analyzed. Both years showed the same pattern and did not differ significantly in relative increase in yolk hormones or estimated onset of incubation before clutch completion (t test, $p > .167$ in all cases). We therefore combined both years in all subsequent analyses.

First, we tested our prediction that the increase of yolk androgens should be steeper with a larger OIC. In line with our hypothesis, there was a significant positive correlation between OIC and the relative increase in yolk testosterone (Pearson correlation: 0.525, $p < .001$; Figure 1). This was not the case for the relative androstenedione increase in relation to the OIC (Pearson correlation: 0.052, $p = .68$).

Furthermore, we hypothesized that variation in OIC should depend on the time span between the laying of the first and the last eggs and on the laying date. In a linear regression, we

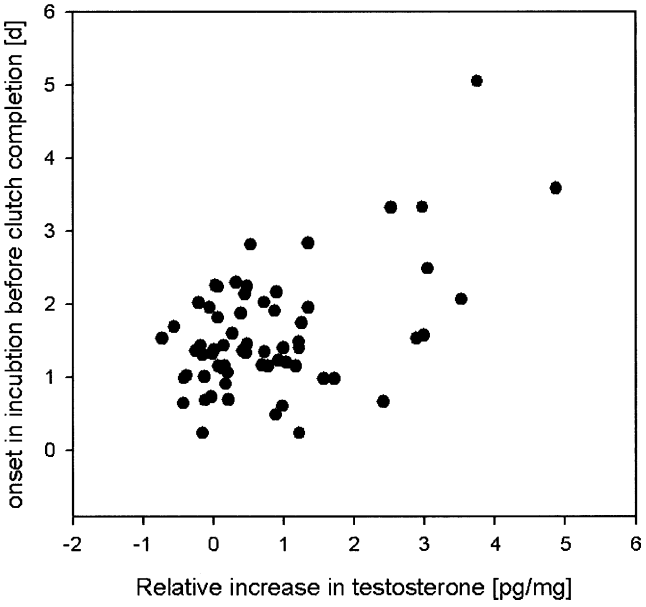

Figure 1
Relative increase in testosterone concentration (pg/mg) between the last-laid and the first-laid eggs, against the duration that incubation has already taken place before clutch completion in days (d).

tested the effect of laying interval and laying date on OIC. Laying interval and laying date were not correlated ($t = 22.737$, df = 63, $p = .795$) and could therefore be used in the same model.

There was a positive correlation between duration of the laying interval and OIC [laying interval of two days (mean): 1.47 days of incubation ($N = 3$); three days: 1.31 ($N = 19$); four days: 1.55 ($N = 28$); five days: 2.03 ($N = 12$); $t = 2.511$, $p = .015$]. Furthermore, OIC increased with a progressing breeding season ($t = 3.353$, $p = .001$; Figure 2).

To investigate the individual contributions of laying date, laying interval, and OIC itself on the steepness of the increase of yolk testosterone, we performed the following linear regression in which the residuals of incubation onset (Res-OIC) on laying date and laying interval as well as the latter two variables were included as predictors for the relative increase in testosterone.

With a longer laying period, the relative increase of testosterone over the laying order was enhanced [laying interval of two days: 0.59 (pg/mg) testosterone increase ($N = 3$); three days: 0.51(pg/mg) ($N = 19$); four days: 0.70 (pg/mg) (28); five days: 1.36 (pg/mg) ($N = 12$); $t = 2.197$, $p = .03$]. The relative increase of testosterone was also positively correlated with the laying date ($t = 2.774$, $p = .007$; Figure 2). Res-OIC values were still positively correlated with the relative increase in testosterone ($t = 3.761$, $p < .001$).

In a third linear regression, we analyzed the relative increase of androstenedione in relation to laying interval, laying date, and the Res-OIC over laying date and duration of the laying period. There was no relationship (linear regression, laying date: $t = -0.901$, df = 53, $p = .251$; duration of the laying period: $t = -0.035$, df = 53, $p = .972$; Res-OIC: $t = 0.465$, df = 53, $p = .465$).

DISCUSSION

Based on the hypothesis that the increase of yolk androgens with laying sequence has a compensatory function in the context of hatching asynchrony, we investigated whether yolk

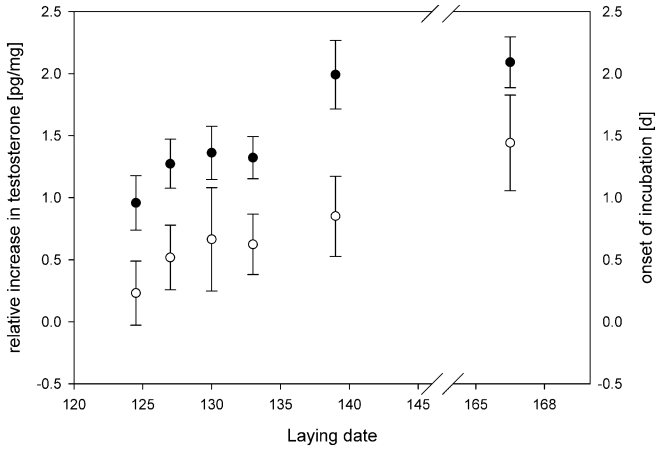

Figure 2
Correlation between laying date (day of the year, subdivided in categories: category 1:124–126, $N = 7$; category 2: 126–128, $N = 11$; category 3: 129–131, $N = 12$; category 4: 132–134, $N = 7$; category 5: 135–142, $N = 14$; category 6 (after flooding): 164–170, $N = 11$). (a) Onset of incubation before clutch completion (open symbols, mean ± SE), and (b) relative increase in testosterone (filled symbols, mean ± SE).

hormone allocation is adjusted to the extent of hatching asynchrony of the clutch. As predicted, the increase in yolk testosterone over the laying sequence was larger when the difference in embryonic development between first- and last-laid eggs, which determines the degree of asynchrony at hatching, was enhanced. Furthermore, the differences in embryonic development and the degree of hatching asynchrony depended on the inter-egg interval and on the time of the year. Also in line with our hypothesis, the allocation of testosterone was enhanced if the laying interval was lengthened and late in the year.

Testosterone accelerates embryonic development, reducing the time difference in hatching between chicks of the same brood (Eising et al., 2001; Lipar, 2001; Lipar and Ketterson, 2000). In addition, it enhances the competitive skills that are particularly important for the youngest chick if the age difference in a brood is large (Eising and Groothuis, 2003; Schwabl, 1993, 1996). Therefore, our findings support the idea that increasing testosterone concentrations with laying order, as reported previously for the black-headed gull (Eising et al., 2001; Groothuis and Schwabl, 2002), plays a compensatory role in the context of hatching asynchrony.

A possible causal explanation for the differential allocation of testosterone may be found in its relationship with prolactin levels. Increasing concentrations of plasma prolactin after the onset of laying enhanced the expression of incubation behavior in American kestrels (Sockman et al., 2000). Enhanced plasma prolactin also increased the deposition of yolk testosterone but not of androstenedione (Sockman et al., 2001). Although the detailed mechanism still has to be discovered, Sockman et al. (2001) suggested that prolactin could influence the activity of aromatase (inhibition), 3-hydroxysteroid dehydrogenase (activation), and 17- hydrosteroid dehydrogenase (activation). This could possibly lead to accumulation of testosterone but evoke only slight changes in androstenedione concentrations. This very likely explains why a relationship between yolk androgens and hatching asynchrony could not be found for wrens (Ellis et al., 2001). Because an overall measure of androgens, rather than testosterone specifically, was used, a potential correlation between testosterone and the degree of hatching asynchrony might be masked by much higher concentrations of androstenedione that did not show a relationship with OIC (e.g., Groothuis and Schwabl, 2002; Schwabl et al., 1997; but see also Schwabl, 1993).

Our results on the relationship between the onset of incubation and androgen allocation in black-headed gulls are consistent with the results of Sockman et al. (2001). As in the kestrel, increased prolactin levels might induce both an early onset of breeding and enhanced testosterone deposition in last-laid eggs. Sockman et al. (2001) also showed a seasonal rise in plasma prolactin that correlated with a seasonal increase in yolk testosterone in the last eggs of a clutch. Also, in our study testosterone increment over the laying sequence was larger with a progressing breeding season. Finally, there is some evidence that a seasonal increase in plasma prolactin reduces the time between the onset of egg laying and the start of incubation during the laying period (Meijer et al., 1990). Indeed, we found that later in the breeding season incubation started significantly earlier compared to the earlier laying dates. Thus, prolactin may play a key role in the adjustment of incubation pattern (and thereby degree of hatching asynchrony) and the deposition of yolk testosterone (compensating effects of hatching asynchrony) to each other. The mechanism by which prolactin could elevate yolk testosterone concentrations is unclear, as prolactin is supposed to be antigonadotropic (e.g., Buntin et al., 1999; Goldsmith, 1983).

Regardless of the precise mechanism, our results support the hypothesis that increasing levels of testosterone over the laying sequence are adjusted to the degree of hatching asynchrony. This supports the idea that yolk testosterone has a compensatory function in the context of hatching asynchrony.

We would like to thank S. Daan, T. Fawcett, S. Marquis, and the referees for providing helpful comments on the manuscript and the farmers of the Linthorst-Homan polder for granting us permission to work on their properties.

REFERENCES

Beukeboom L, Dijkstra C, Daan S, Meijer T, 1988. Seasonality of clutch size determination in the kestrel, *Falco tinnunculus*: an experimental study. Ornis Scand 19:41–48.

Bollinger PB, Bollinger EK, Malecki RA, 1990. Tests of the three hypotheses of hatching asynchrony in the common tern. Auk 107: 696–706.

Brouwer A, Spaans AL, 1994. Egg predation in the Herring Gull *Larus argentatus*: why does it vary so much between nests? Ardea 82: 223–230.

Buntin JD, Advis JP, Ottinger MA, Lea RW, Sharp PJ, 1999. An analysis of physiological mechanisms underlying the antigonadotropic action of intracranial prolactin in ringdoves. Gen Comp Endocrinol 114:97–107.

Clark AB, Wilson, DS, 1981. The onset of incubation in birds. Am Nat 125:603–611.

Courtney P, 1979. Seasonal variation among intra-clutch hatching intervals among common tern *Sterna hirundo*. Ibis 121:207–211.

Cramp S, Simmons, KEL (eds), 1983. Handbook of the birds of Europe, the Middle East and North Africa: the birds of the Western Paleartic. Volume III: waders to gulls. Oxford: Oxford University Press.

Dunlop E, 1910. On incubation. British Birds 4:137–145.

Eising CM, Eikenaar C, Schwabl H, Groothuis TGG, 2001. Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development. Proc R Soc Lond B 268:839–846.

Eising CM, Groothuis TGG, 2003. Yolk androgens and begging behaviour in black-headed gull chicks: an experimental field study. Anim Behav 66:1027–1034.

Elf PK, Fivizanni AJ, 2002. Changes in sex steroid levels in yolks of the Leghorn chicken, *Gallus domesticus*, during embryonic development. J Exp Zool 293:594–600.

Ellis LA, Borst DW, Thompson CF, 2001. Hatching asynchrony and maternal androgens in egg yolks of house wrens. J Avian Biol 32: 26–30.

Forbes LS, Thornton S, Glassey B, Forbes M, Buckley N, 1997. Why parent birds play favourites. Nature 390:351–352.

French JB Jr, Nisbet ICT, Schwabl, H, 2001. Maternal steroids and contaminants in common tern eggs: a mechanism of endocrine disruption? Comp Biochem Physiol 128:91–98.

Gil D, Graves J, Hazon N, Wells A, 1999. Male attractiveness and differential testosterone investment in zebra finch eggs. Science 286:126–128.

Godsave SF, Lohmann R, Vloet RPM, Gahr M, 2002. Androgen receptors in the embryonic zebra finch hindbrain suggest a function for maternal androgens in the perihatching survival. J Comp Neurol 453:57–70.

Goldsmith AR, 1983. Prolactin in avian reproductive cycles. In: Hormones and behaviour in higher vertebrates (Balthazart J, Pröve E, Gilles R, eds). Berlin: Springer Verlag; 375–387.

Graves J, Whiten A, Henzi P, 1984. Why does the herring gull lay three eggs? Anim Behav 32:798–805.

Groothuis TGG, Schwabl H, 2002. The influence of laying sequence and habitat characteristics on maternal yolk hormone levels. Funct Ecol 16:281–289.

Hebert PN, McNeil R, 1999. Hatching asynchrony and food stress in Ring-billed gulls: an experimental study. Can J Zool 77:515–523.

Lack D, 1947. The significance of clutch size. Ibis 89:302–352.

Lipar JL, 2001. Yolk steroids and the development of the hatching muscle in nestling European starlings. J Avian Biol 32:231–238.

Lipar JL, Ketterson ED, 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-winged blackbird *Agelius phoeniceus*. Proc R Soc Lond B 267: 2005–2010.

Lipar JL, Ketterson ED, Nolan V, 1999. Intra-clutch variation in testosterone contents of red-winged blackbirds eggs. Auk 116:231–235.

MacRoberts MH, MacRoberts BR, 1972. The relationship between laying date and incubation period in Herring and lesser black-backed gulls. Ibis 121:93–97.

Mead PS, Morton ML, 1985. Hatching asynchrony in the mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*): a selected or incidental trait? Auk 102:781–792.

Meijer T, Daan S, Hall M, 1990. Family planning in the kestrel (*Falco tinnunculus*): the proximate control of co-variation of laying date and clutch size. Behaviour 114:117–136.

Mock DW, Drummond H, Stinson CH, 1990. Avian siblicide. Am Sci 78:438–449.

O'Connor RJ, 1978. Brood reduction in birds: selection for fratricide, infanticide and suicide? Anim Behav 26:79–96.

Parsons J, 1972. Egg size, laying date and incubation period in the herring gull, *Larus argentatus*. Ibis 114:536–541.

Parsons J, 1976. Factors determining the number and size of eggs laid by the herring gull. Condor 78:481–492.

Ricklefs RE, 1987. Comparative analysis of avian embryonic growth. J Exp Zool Suppl 1:309–323.

Royle NJ, Surai PF, Hartley IR, 2001. Maternal derived androgens and antioxidants in bird eggs: complementary but opposing effects? Behav Ecol 12:381–385.

Schwabl H, 1993. Yolk is a source of maternal testosterone for developing birds. Proc Natl Acad Sci USA 90:11446–11450.

Schwabl H, 1996. Maternal testosterone in the egg enhances postnatal growth. Comp Biochem Physiol 114:271–276.

Schwabl H, Mock DW, Gieg JA, 1997. A hormonal mechanism for parental favouritism. Nature 386:231.

Sharp PJ, Scanes CG, Williams JB, Harvey S, Chadwick A, 1979. Variations in concentrations of prolactin, luteinizing hormone, growth hormone and progesterone in the plasma of broody bantams (*Gallus domesticus*). J Endocrinol 80:51–57.

Sockman KW, Schwabl H, Sharp PJ, 2000. The role of prolactin in the regulation of clutch size and onset of incubation behavior in the American kestrel. Horm Behav 38:168–176.

Sockman KW, Schwabl H, Sharp PJ, 2001. Regulation of yolk-androgen concentrations by plasma prolactin in the American kestrel. Horm Behav 40:462–471.

Stockland JN, Amudsen T, 1988. Initial size hierarchy in broods of the shag: relative significance of egg size and hatching asynchrony. Auk 107:359–366.

Stoleson SH, Beissinger SR, 1995. Hatching asynchrony and the onset of incubation in birds, revisited: when is the critical period? In: Current ornithology (Power DM, ed). Vol. 12, New York: Plenum; pp 191–271.

Webb DR, 1987. Thermal tolerance of avian embryos: a review. Condor 89:874–898.

Woods JE, Simpson RM, Moore PL, 1975. Plasma testosterone levels in the chick embryo. Gen Comp End 27:543–547.